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# Higher bone resorption excretion in South Asian women vs White Caucasians and increased bone loss with higher seasonal cycling of vitamin D: results from the D-FINES cohort study

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**Abbreviations**

uNTX: Urinary collagen type 1 cross-linked N-telopeptide

D-FINES: Diet, Food Intake, Nutrition and Exposure to the Sun in Southern England

## Disclosures:

SL-N discloses that she is research director of D3-TEX Ltd.

## Highlights:

Few data exist on bone resorption in Western dwelling South Asian women.

Bone resorption was assessed in premenopausal and postmenopausal South Asian and Caucasian women living in Southern England.

Premenopausal South Asians had higher bone resorption than same-age Caucasians, and had similar bone resorption to postmenopausal South Asians.

Non-linear mixed modelling analysis suggested that individuals with a higher seasonal change in 25(OH)D showed increased levels of bone resorption.

Comparison of effect sizes for seasonal change and average 25(OH)D suggested seasonal change was the stronger predictor of bone resorption.

**Keywords:**

25-hydroxyvitamin D, ethnicity, bone resorption, season, urinary n-telopeptide

**Abstract**

Few data exist on bone turnover in South Asian women and it is not well elucidated as to whether Western dwelling South Asian women have different bone resorption levels to that of women from European ethnic backgrounds. This study assessed bone resorption levels in UK dwelling South Asian and Caucasian women as well as evaluating whether seasonal variation in 25-hydroxyvitamin D [25(OH)D] is associated with bone resorption in either ethnic group.

Data for seasonal measures of urinary N-telopeptide of collagen (uNTX) and serum 25(OH)D were analysed from n=373 women (four groups; South Asian postmenopausal n=44, South Asian premenopausal n=50, Caucasian postmenopausal n=144, Caucasian premenopausal n =135) (mean (± SD) age 48 (14) years; age range 18-79 years) who participated in the longitudinal D-FINES (Diet, Food Intake, Nutrition and Exposure to the Sun in Southern England) cohort study (2006-2007).

A mixed between-within subjects ANOVA (n=192) showed a between subjects effect of the four groups ($P<0.001$) on uNTX concentration, but no significant main effect of season ($P=0.163$). Bonferroni adjusted Post hoc tests ($P\leq0.008$) suggested that there was no significant difference between the postmenopausal Asian and premenopausal Asian groups. Season specific age-matched-pairs analyses showed that in winter ($P=0.04$) and spring ($P=0.007$), premenopausal Asian women had a 16 to 20 nmol BCE/mmol Cr higher uNTX than premenopausal Caucasian women.

The (amplitude/mesor) ratio (i.e. seasonal change) for 25(OH)D was predictive of uNTX, with estimate(SD)=0.213 (0.015) and 95% CI (0.182, 0.245; $P<0.001$) in a non-linear mixed model (n=154). This showed that individuals with a higher seasonal change in 25(OH)D, adjusted for overall 25(OH)D concentration, showed increased levels of uNTX. Although the effect size was smaller than for the amplitude/mesor ratio, the mesor for 25(OH)D concentration was also predictive of uNTX, with estimate(SD)= -0.035 (0.004), and 95% CI (-0.043, -0.028); $P<0.001$).

This study demonstrates higher levels of uNTX in premenopausal South Asian women than would be expected for their age, being greater than same-age Caucasian women, and similar to postmenopausal Asian women. This highlights potentially higher than expected bone resorption levels in premenopausal South Asian women which, if not offset by concurrent increased bone formation, may have future clinical and public health implications which warrant further investigation. Individuals with a larger seasonal change in 25(OH)D concentration showed an increased bone resorption, an association which was larger than that of the 25(OH)D yearly average, suggesting it may be as important clinically to ensure a stable and steady 25(OH)D concentration, as well as one that is high enough to be optimal for bone health.

## 1.0 Introduction

Despite the hypothetical increase in osteoporotic fracture risk due to smaller bone size in postmenopausal South Asian women [1] few studies have assessed bone health in this population group. Also, recent epidemiological research has suggested that US dwelling South Asian women have an increased prevalence of femoral neck osteoporosis [2] but have no differences in wrist fracture rates [2] compared with same-age Caucasian women, suggesting that bone health in South Asian women needs further elucidation.

Bone turnover markers are a known predictor of fracture risk [3] as well as giving valuable insight into the bone turnover related mechanisms underlying inter-individual differences in bone structure. However, there is a lack of information on bone turnover rates in South Asian women, either western dwelling, or dwelling in country of origin. Only three studies, to the authors' knowledge [4-6] have reported levels of bone turnover activity in migrant South Asian women living in Western countries. These studies found either no difference in bone turnover between South Asian and Caucasian populations [4, 5] or no difference between older and younger South Asian women [6], the latter result of which is of concern considering the premenopausal status of the younger women. These cross-sectional studies highlight important similarities and differences between bone turnover in western dwelling South Asian and Caucasian women. However, there is still a need for studies to assess longitudinal change in bone turnover markers over the course of at least one year in South Asian women to see if these ethnic and age differences in bone turnover vary by season, and if so how.

There is also a need to understand the relationship between vitamin D status and bone resorption, specifically how this relationship may be mediated by season, menopausal status or ethnicity. It is known that adequate vitamin D status is important for bone health, with associations between bone turnover and 25(OH)D status being found in Caucasian populations in some studies [7, 8]. However, it is relatively unknown as to whether there is an association between 25(OH)D status and bone turnover in South Asian women, with the only published study (UK) finding no cross-sectional relationship [4]. The known lower 25(OH)D status in many Western dwelling South Asian women, as compared to Caucasian women [4, 9-11], suggests theoretically that the relationship between 25(OH)D and bone turnover may be stronger in South Asian women, but more research is required to establish whether this is the case.

In addition to that of overall average yearly 25(OH)D concentrations, degree of annual fluctuation in 25(OH)D could also theoretically influence bone health. This is because changes in 25(OH)D substrate have a large impact on the activity of the 1-hydroxylase enzyme, which is one of the main hydroxylase enzymes controlling 1,25-dihydroxyvitamin D [1,25(OH)$_2$D] levels, and at physiological concentrations of 25(OH)D is working well below its Michaelis-Menten constant [Km]. Large changes in the activity of the 1-hydroxylase enzyme could occur as a result of large seasonal fluctuations in 25(OH)D and have an impact on 1,25(OH)$_2$D concentration [12]. Some previous research studies in Caucasian populations have found no relationship between seasonal fluctuation of 25(OH)D and bone resorption [13] or no acute effect of vitamin D supplementation during winter time (to blunt the seasonal rhythm) on bone resorption or formation markers [14]. However, data from South Asian and Caucasian populations in the D-FINES (Vitamin **D**, **F**ood **I**ntake, **N**utrition and **E**xposure to

**S**unlight in Southern England) cohort study suggested that a larger change in 25(OH)D over the course of a year was associated with increased bone turnover [uCTX], in comparison to those with a smaller change in 25(OH)D [15]. However, subject numbers were relatively small and only sCTX levels were measured.

The present work is a larger follow-up analysis of the D-FINES cohort. We investigate whether there is a difference (within and between seasons) in bone resorption (uNTX) between Caucasian and South Asian women, when adjusted for confounding factors, as well as examining whether there is a relationship between serum 25(OH)D concentration and bone resorption in either ethnic group. We also determine the relative abilities of seasonal change and average 25(OH)D to predict bone resorption over the course of one year, using a non-linear mixed modelling approach. Based on the results of our previous analysis [15], as well as the above literature, it was hypothesised that South Asian women would have increased bone resorption as compared with their Caucasian counterparts. It was also predicted there would be an association between 25(OH)D status and bone resorption within seasons, and that individuals showing a high degree of seasonal fluctuation in 25(OH)D over the course of the year would also show higher bone resorption than those with a lower degree of seasonal fluctuation in 25(OH)D.

## 2.0 Materials and Methods

### 2.1 Study Design

Subjects had taken part in the 2006-2007 UK Food Standards Agency D-FINES study (Project N05064) [9], whereby they attended the University of Surrey once per season for one year (four visits) for sampling of blood for measurement of 25(OH)D status and of urine for measurement of uNTX, as well as for collection of anthropometric, dietary and lifestyle information. Data from *n* =373 women (South Asian postmenopausal n=44, South Asian premenopausal n=50, Caucasian postmenopausal n=144, Caucasian premenopausal *n* =135) was available for analysis. Menopausal status was derived from the date of the last menstrual period (self-reported), with postmenopausal status being defined as the participant having had no menstrual periods for >3 months. Any postmenopausal women who had used hormone replacement therapy in the last year were excluded from the study. Further details regarding the D-FINES study, including subject recruitment, study procedure, other exclusion criteria and background information have been previously reported [9]. In accordance with the ethical standards laid down in the 1964 Declaration of Helsinki, ethical reviews were obtained from relevant Research Ethics Committees (National Health Service NHS REC 06/Q1909/1, and University of Surrey EC/2006/19/SBMS). Written, informed consent was obtained from all participants.

### 2.2 Biochemical Measurements

Measurement of uNTX was undertaken at the University of Sheffield (Metabolic Bone Centre, Northern General Hospital, Sheffield, UK), funded by a grant (number 225) from the National Osteoporosis Society (2011). Measurements were undertaken using the automated

Vitros ECi, Ortho Clinical Diagnostics analyser (Rochester NY, USA) and were adjusted for creatinine (Cr) excretion. Serum 25(OH)D was measured by the Vitamin D Research Group at the University of Manchester, using the manual IDS enzyme immunoassay (Immunodiagnostic Systems Ltd, Boldon, Tyne and Wear, UK) with inter-assay and intra-assay coefficients as described previously [9].

### 2.3 Statistical methods, including Non-Linear Mixed Modelling Analysis

Ethnic and menopausal status differences in uNTX were analysed using mixed between-within subject analysis of variance (ANOVA; women with all four uNTX measurements). Within-season ANCOVA was also conducted (using all women with at least one uNTX measurement) to allow for the analysis of data from women who did not have full data for uNTX for all seasons (to reduce the impact of attendance bias). For the premenopausal women only, an age-matched analysis was also undertaken due to a biologically significant age difference between the two premenopausal groups (mean (SD) for age: 34.0 (5.6) years in the Caucasians and 39.0 (8.5) years in the South Asians). The premenopausal South Asian women were individually matched to one same-age (to the exact year) premenopausal Caucasian. When there were multiple Caucasian women of the same age available, SPSS was used to randomly choose one Caucasian subject to be matched to each South Asian subject. Finally, to assess the relationship between 25(OH)D and uNTX Pearson's bivariate and partial correlations were conducted. The statistical package SPSS (v21, 2013, Chicago IL) was used to analyse baseline participant data and conduct the ANOVA, ANCOVA and correlation analyses. When statistically significant sphericity ($P>0.05$) was present in the ANOVA analyses the Greenhouse-Geisser estimate was used.

Non-linear mixed modelling (NLM) was used to assess whether individuals with increased seasonal change in 25(OH)D would show increased bone resorption (uNTX). This modelling procedure has been used previously in the D-FINES cohort and described in detail [15]. In summary, the NLMIXED procedure, of the SAS (SAS Institute, Cary, NC, USA) software suite was used to conduct the non-linear mixed modelling analysis. BMI and ethnic-menopausal status were considered potential confounders, and were retained in the model at all times. Regression parameters significantly different from zero within the limits of the conventional 95% confidence interval were deemed statistically significant. The flow of participants through the various analyses in the manuscript is illustrated in Supplementary Figure 1.

## 3.0 Results

### 3.1 Participant Characteristics

Results are presented as mean (SD) unless otherwise stated. Supplementary Table 1 shows the baseline characteristics of the D-FINES participants included in the ANOVA analysis (n=192) including 25(OH)D and uNTX concentration in each season and anthropometric information. Mean (SD) for age was 52 (13) years and dietary calcium intake was 859 (269) mg/d. Mean (SD) for BMI was 26.2 (4.5) Kg/m$^2$, thus the participants on average were classified as overweight. Depending on season, concentrations of 25(OH)D ranged from 41.4-62.9 nmol/L and for uNTX ranged from 54.0-55.5 nmol BCE/mmol Cr. Supplementary Table 2 shows background information for the subset of the women who had complete data for all relevant variables for the NLM analysis (n=154).

### 3.2 Levels of bone resorption markers by ethnic menopausal group

#### 3.2.1 uNTX by ethnic-menopausal group

uNTX concentrations for the four groups of women (all women with at least one uNTX measurement) are shown in Figure 1. Our data show ranges of uNTX concentrations of 48-52 nmol BCE/mmol Cr for premenopausal South Asian women and 48-55 nmol BCE/mmol Cr for postmenopausal South Asian women, depending on season. This is in contrast to 40-42 nmol BCE/mmol Cr in Caucasian premenopausal women, and 62-66 nmol BCE/mmol Cr in Caucasian postmenopausal women (Table 1, Figures 1A-D). Of note, there was no difference in uNTX between the premenopausal South Asian women and the postmenopausal South

Asian women in any season of the year (Figure 1B). This is in contrast to premenopausal Caucasian women who have lower bone resorption than postmenopausal Caucasian women all year around (Figure 1A). Postmenopausal Asian women had a lower uNTX than postmenopausal Caucasian women in autumn only (Figure 1C) and there was no difference in uNTX between the two premenopausal groups in any season (Figure 1D).

FIGURE 1A-D ABOUT HERE

TABLE 1 ABOUT HERE

### 3.2.2 ANOVA analysis

A mixed between-within subjects ANOVA, adjusting for BMI and dietary calcium as covariates (n=192) showed no significant main effect of season (P=0.16) on uNTX, as well as no interaction effect between season and group (P=0.61). However, there was a significant between subjects effect of the four groups (P<0.001) (Figure 2). Observed power for the between-subjects, season effect and season-group interaction were 99.9%, 43.0% and 37.6% respectively. Bonferroni adjusted post hoc tests showed that the only statistically significant differences were between the two Caucasian groups (P<0.001) with a non-statistically significant trend for a difference between the Caucasian premenopausal and postmenopausal Asian groups (P=0.07) and between the two premenopausal groups (P=0.07).

### 3.2.3 Within-season ANCOVA analysis

Season-specific ANCOVA, controlling for BMI and dietary calcium as covariates, showed that there was a statistically significant overall group difference in uNTX in Summer ($P<0.001$, n=286); Autumn ($P<0.001$ n=264) Winter ($P<0.001$, n=235) and Spring ($P<0.001$, n=230). Bonferroni adjusted post hoc tests showed significant differences between the Caucasian premenopausal and Asian postmenopausal groups in summer (P=0.015), winter (P=0.05) and spring (P=0.016). In autumn, winter and spring ($P<0.001$) there were significant differences between the two Caucasian groups. There was a non-statistically significant trend for a difference between the Caucasian postmenopausal and Asian premenopausal groups in summer (Summer P=0.085) and between the two premenopausal groups in autumn (P=0.062).

### 3.2.4 Premenopausal women paired for exact age

Paired t-tests, using age-matched pairs, showed that there was no significant difference in uNTX between the two premenopausal groups in autumn (P=0.27 n=15 pairs) (Figure 3). However, Asians had a significantly higher uNTX in winter by a mean of 16 nmol BCE/mmol Cr (P=0.007 n=16 pairs) and in spring by a mean of 20 nmol BCE/mmol Cr (P=0.04, n=15 pairs), with a non-statistically significant trend for a statistically significantly higher uNTX in Asians by a mean of 9 nmol BCE/mmol Cr in Summer (P=0.08 n=25 pairs)(Figure 3).

FIGURE 3 ABOUT HERE

### 3.3 Associations between 25(OH)D and uNTX

Within-season partial correlations (controlling for BMI, ethnicity and menopausal status) showed no statistically significant associations between 25(OH)D and uNTX in Autumn (r= -

0.08, P=0.23 n=263) Winter (r= -0.10, P=0.14 n=221) or Spring (-0.10, P=0.14 n=219) with a weak, non-statistically significant trend for an association in Summer (r= -0.10 P=0.07 n=322). For ethnic specific estimates (controlling for BMI and reproductive status), there were statistically significant correlations in the Asian group in the Summer (r= -0.24, P=0.04, n=71) only. For the postmenopausal South Asians group (controlling for BMI) there were statistically significant associations of moderate magnitude in Summer (r= -0.36, P=0.04 n=33), and a trend for an association in Winter (r= -0.35, P=0.09 n=23), but there were no statistically significant results or trends for the other three groups in any season (P>0.05).

**3.4 Non-Linear Mixed Modelling**

Table 2 illustrates the regression analysis, including the effect sizes for the main model parameters after adjustment for confounders (BMI and ethnic/menopausal group). The regression coefficient (SE) for the amplitude/mesor ratio of 25(OH)D was 0.213 (0.015) with a 95% confidence interval (0.182, 0.245; P<0.001) and an effect size of 13.4. Hence the amplitude/mesor parameter for 25(OH)D was a significant predictor of uNTX concentration. The regression coefficient (SE) for the mesor level of 25(OH)D was -0.035 (0.004) with a 95% confidence interval of (-0.043, -0.028; P<0.001) and an effect size of 9.1. This indicates that the mesor for 25(OH)D was a significant negative predictor of uNTX concentration. In addition, the effect size for the mesor was smaller than that for the amplitude/mesor ratio.

TABLE 2 ABOUT HERE

## 4.0 Discussion

### 4.1 Summary of findings

We found significant differences between the two Caucasian groups in all seasons, with postmenopausal Caucasians having a 1.5 fold higher uNTX across the year than did the premenopausal Caucasians. However, there was no difference in uNTX between the premenopausal and postmenopausal South Asians in any season. Although there was a trend for the postmenopausal South Asians to have a lower uNTX than postmenopausal Caucasian women, this was only statistically significant in autumn. Therefore, the smaller difference seen between the Asian groups compared with the Caucasian groups is not simply due a relatively lower uNTX in the older Asians than in the older Caucasians. Moreover, the matched pairs analysis showed a significant difference between the two premenopausal groups, with premenopausal Asians 16-20 nmol BCE/mmol Cr higher (in winter and spring) once the two groups were exactly age matched.

### 4.2 Ethnicity, 25(OH)D and bone resorption

In terms of previous literature, our study findings support those of Lowe et al (2010) who found no difference in either the levels of bone resorption [serum c-telopeptide; sCTX] or the levels of bone formation [serum procollagen type 1 N propeptide; sP1NP] between UK dwelling South Asian and Caucasian postmenopausal women [4]. However, higher bone formation [serum BAP concentration] was found in the South Asian women [4]. No relationship was found between 25(OH)D status and bone resorption levels in either ethnic group [4], which is in agreement with the findings of our current study. Our results also support those of a vitamin D supplementation study which measured bone turnover in New

Zealand dwelling South Asian women and found similar baseline levels of uNTX (and serum Osteocalcin [sOCN]) in the premenopausal and postmenopausal groups [6]. This suggests similar bone resorption and formation irrespective of menopausal status in South Asian women.

It is unclear what the mechanism is behind our finding of higher bone resorption in premenopausal South Asians compared with their same-age Caucasian peers. In premenopausal women, inter-individual variation in bone turnover marker concentrations have been associated with contraceptive pill use [16, 17], exercise [16], smoking status [16], and 25(OH)D concentration [16] in some studies, but not in others [18]. In our n=363 sample (n=177 premenopausal), 16% of premenopausal Caucasians reported that they were smokers compared with 2% of the premenopausal South Asians, and 9% of premenopausal Caucasians vs. only 2% of premenopausal Asians were on the oral contraceptive pill. This suggests a higher presence of smoking and contraceptive pill use in the premenopausal Caucasians. However, average minutes of physical activity per day (walking and cycling combined) were around 50 mins per day in premenopausal Asians, and around 40 mins per day in premenopausal Caucasians (data not shown), so self-reported exercise levels were similar between the two ethnic groups, as was BMI.

However, the premenopausal South Asian women in our study were more deficient in 25(OH)D and had higher parathyroid hormone (PTH) levels than the premenopausal Caucasian women throughout the year, (data previously published [9]). In previous research examining premenopausal South Asians residing in Pakistan, secondary hyperparathyroidism due to vitamin D deficiency has been shown to explain high bone resorption levels (uNTX)

[19]. Still, we found little evidence of a relationship between 25(OH)D status and uNTX concentration in either ethnic group in our study, despite 25(OH)D concentrations being lower in our premenopausal South Asian women [mean(SD): 30(15) nmol/L] than those of Khan et al. (2013)[19] [mean(SD): 55(54) nmol/L].

It will be important in future research to assess overall levels of bone formation in younger and older South Asian women, as it is possible that an increase in bone resorption may be coupled with increased bone formation. In this case, higher bone resorption in these women may be less detrimental to bone health than would be the case if bone formation was low. High bone formation markers have been seen in South Asian women in comparison with Caucasian women in other research studies [4, 5] as well as similar levels of bone formation markers between postmenopausal and premenopausal South Asian women [6]. Moreover, it is not clear as to whether our findings of increased bone resorption in premenopausal South Asian women show pathology or physiology.

### 4.3 Predictive ability of 25(OH)D seasonal change and yearly average in explaining bone resorption

We found that seasonal change in 25(OH)D was a positive predictor of uNTX, supporting our original hypothesis of a positive association between seasonal fluctuation in 25(OH)D and bone resorption levels. This result indicates that those individuals with a higher seasonal change in 25(OH)D have a higher uNTX, which suggests increased bone resorption. It also supports the hypothesis that a larger degree of seasonal changes in 25(OH)D might lead to a health detriment for some outcomes [20]. As found in our smaller scale previous work with the bone marker sCTX [15], in the current study average concentration of 25(OH)D had a smaller effect size than did seasonal fluctuation in explaining uNTX. This study supports that reported previously [15] which suggests that seasonal variation in 25(OH)D concentration is at least as important as average yearly concentration of 25(OH)D, perhaps even more so. However it contradicts previous research in postmenopausal Caucasian women, a Scottish study found no association between degree of seasonal change in 25(OH)D and bone resorption levels [uCTX] [13]. Also, an Irish intervention study of young adults found no effect of wintertime supplementation (15 micrograms per day; 22 weeks) of vitamin D on subsequent bone turnover [14], suggesting that supressing the winter-time reduction in 25(OH)D status did not benefit bone health, at least in the short term.

**4.4 Implications of this work**

Our findings highlight that in addition to having a slender bone size, premenopausal South Asian women have also higher bone resorption than would be expected for their age. Further research is urgently required to fully elucidate the reason for this increased bone resorption, and to assess whether bone formation is also increased, or not, in premenopausal South Asian women. The clinical implications of this work are that South Asian women may need to be targeted for bone health relevant interventions at a much younger age. Also, reference ranges for bone markers may need to take account of differing ethnic backgrounds. In addition, the long-term effects of seasonal fluctuation in vitamin D status now need to be assessed, in order to determine whether there are longer term effects on bone health, including effects on bone structure and clinical end points such as fracture risk. If a relevant long-term effect is seen, then the clinical implications are that that it might be of benefit to only take vitamin D supplements in the wintertime, not all year around, thus ensuring a blunting of the amount of seasonal change in 25(OH)D.

### 4.5 Strengths and Limitations

Our study adds significantly to the previous literature, as it is the first study to assess differences in bone resorption between premenopausal Caucasians and premenopausal South Asians, as well as the first to assess the relationship between 25(OH)D status and bone resorption in premenopausal South Asian women. Thus, it is the first to report that premenopausal South Asians have significantly higher bone resorption levels in winter and spring than do same-age Caucasian women, and that there is no significant relationship between 25(OH)D status and bone resorption in premenopausal South Asian women. Nonetheless, it must be borne in mind that these data came from only Caucasian and South Asian women, and may not be applicable to other ethnic groups, due to potential ethnic differences in vitamin D metabolism (e.g genetics affecting activity of vitamin D hydroxylase enzymes or vitamin D binding protein activity [21, 22]). It is important to note that the sample size was very small for the South Asian groups in some of the analyses, particularly for the premenopausal women. This was due to few of the group having full data for all seasons (e.g. many had at least one season missing, prohibiting entry into the ANOVA and the non-linear mixed modelling). Also, there is a clear need for the concurrent measurement of a bone formation marker to assess overall bone turnover, as high bone resorption in the premenopausal South Asian women would not necessarily be detrimental to bone health if coupled with high bone formation.

## 5.0 Conclusions

Our results add to that of previous studies of bone markers in South Asian women by introducing a longitudinal perspective over the course of the year and is the first study to our knowledge to examine bone changes in this ethnic group in this way. We have shown that premenopausal South Asian women do not differ from postmenopausal South Asian women in bone resorption levels in any season of the year. This is in contrast to premenopausal Caucasian women who have lower bone resorption than postmenopausal Caucasian women all year around, reaching a statistically significant difference in autumn and winter. The increased bone resorption observed in the premenopausal South Asian women, relative to their same age Caucasian counterparts, warrants further investigation. This is because if increased bone resorption is not offset by increased bone formation, it may lead to a detriment to bone health.

In addition, this work has been the first to show (in a large sample size) that seasonal fluctuation in vitamin D status is associated with bone resorption markers in UK dwelling premenopausal and postmenopausal women. The level of 25(OH)D corresponding to sufficiency and prevention of chronic disease has been long debated in scientific literature but is currently set at 50nmol/L in the US/Canada [23]. Our findings are of importance as they suggest that 25(OH)D may also need to be stable over the course of the year, and that stability of vitamin D levels may be as important as actual average concentration of vitamin D. Further research is now required to assess the levels of bone formation in South Asian women and whether bone formation levels vary by degree of seasonal fluctuation of 25(OH)D.

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Author contributions: 1) conception and design ALD, SAL-N, KH, RE; 2) acquisition of data ALD KHH FR JLB FG SAL-N;  3) analysis and interpretation of data SAL-N, KH, ALD RV JLB FG TRH JH RE SJ; 4) ALD drafted the manuscript; 5) All authors revised the manuscript critically for important intellectual content.

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## Dedication

This paper is dedicated to Mr John Pheasant, Practice Manager at Thornton Heath Medical Centre, London who helped with the study recruitment and who sadly died in 2008.

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**Figure Legends**

Figure 1 Comparison of unadjusted uNTX data within ethnic groups, women with 1+ seasons (n=344 at baseline (summer)): (A) Two Caucasian groups (B) Two South Asian groups (C) Two postmenopausal Groups (D) Two premenopausal groups

Figure 2 Comparison of all four groups by season- women with full data for uNTX in all 4 seasons only (n=192). Bonferroni adjusted post hoc tests showed the only statistically significant group difference was between the two Caucasian groups ($P<0.001$).

Figure 3 Premenopausal matched pairs (women with uNTX for 1+ seasons) A:Summer (n=25 pairs), B:Autumn (n=15 pairs), C:Winter (n=16 pairs), D:Spring (n=15 pairs)

## Tables

**Table 1 N-telopeptide values for all women who had uNTX data for one or more seasons (n=363)**

| | Cauc Post | | | Cauc Pre | | | Asian Post | | | Asian Pre | | | P¥ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **N** | **Mean** | **SD** | **n** | **Mean** | **SD** | **n** | **Mean** | **SD** | **n** | **Mean** | **SD** | |
| Age (years) | 143 | 61.4a | 4.5 | 132 | 34.0abc | 5.6 | 43 | 59.6b | 5.9 | 45a | 39.0bc | 8.5 | <0.001 |
| Body Mass Index (BMI; kg/m$^2$) | 143 | 26.5b | 5.0 | 132 | 25.4a | 4.5 | 41 | 30.0abc | 6.2 | 45 | 26.3c | 4.5 | <0.001 |
| Index of Multiple Deprivation (IMD) | 143 | 16.7 | 9.6 | 132 | 16.0 | 9.1 | 43 | 16.0 | 13.0 | 45 | 12.7 | 8.0 | 0.123 |
| Summer uNTX (BCE/mmol creatinine) | 137 | 65.8ab | 39.5 | 126 | 41.1a | 20.2 | 40 | 55.1 | 29.4 | 41 | 50.5b | 31.3 | <0.001 |
| Autumn uNTX (BCE/mmol creatinine) | 132 | 61.6ab | 28.2 | 105 | 41.9a | 17.8 | 34 | 47.9b | 23.9 | 22 | 48.5 | 32.2 | <0.001 |
| Winter uNTX (BCE/mmol creatinine) | 123 | 65.9ab | 37.5 | 83 | 39.6a | 13.5 | 30 | 52.7 | 24.3 | 25 | 47.6b | 26.4 | <0.001 |
| Spring uNTX (BCE/mmol creatinine) | 128 | 62.7a | 33.3 | 76 | 40.4a | 17.2 | 27 | 52.9 | 24.0 | 22 | 51.7 | 29.6 | <0.001 |
| Summer 25(OH)D nmol/L | 138 | 64.0abc | 19.1 | 125 | 72.5ade | 26.3 | 37 | 28.4bd | 11.6 | 42 | 29.6ce | 14.8 | <0.001 |
| Summer Ca intake (mg/d) | 137 | 864 | 285 | 111 | 834 | 319 | 33 | 801 | 254 | 22 | 717 | 266 | 0.153 |
| | | | | | | | | | | | | | |
| | **N** | **Median** | **IQR** | **n** | **Median** | **IQR** | **n** | **Median** | **IQR** | **n** | **Median** | **IQR** | **P¥-** |
| Summer sPTH (pmol/L)≠ | 138 | 2.85a | 1.5 | 125 | 2.30abc | 1.65 | 36 | 3.50b | 1.48 | 42 | 3.20c | 2.02 | <0.001 |
| Summer VitD intake (micrograms/d)≠ | 137 | 2.45ab | 2.65 | 111 | 1.86a | 2.19 | 33 | 1.39b | 1.34 | 22 | 1.49 | 1.85 | 0.002 |
| | | | | | | | | | | | | | |

Cauc Pre=premenopausal Caucasian, Cauc Post=postmenopausal Caucasian, Asian Pre=premenopausal Asian, Asian Post=postmenopausal Asians. ¥One Way ANOVA, with Tukey's HSD Posthoc tests; like superscripts are significantly different comparisons. ≠log transformed prior to statistical analysis.

**Table 2- Relevant non-linear modelling parameter statistics for uNTX (n=154[≠])**

| Parameter | Estimate | SE | 95% CI | P | Effect size ¥ |
|---|---|---|---|---|---|
| Indicator (0 1) variable for (PRE C) v (POST C))* | -0.124 | 0.93 | -1.954, 1.706 | 0.894 | 0.13 |
| Indicator (0 1) variable for (POST SA)v(POST C)* | 0.316 | 1.88 | -3.380, 4.011 | 0.867 | 0.17 |
| Indicator (0 1) variable for (PRE SA)v(POST C)* | 0.303 | 2.37 | -4.334, 4.940 | 0.898 | 0.13 |
| BMI** (Body mass index) $kg/m^2$ | 0.073 | 0.04 | -0.010, 0.157 | 0.085 | 1.72 |
| 25(OH)D regression coefficient | -0.035 | 0.004 | -0.043,-0.028 | <0.001 | 9.13 |
| 25(OH)D Ratio (amplitude/mesor) | 0.213 | 0.015 | 0.182, 0.245 | <0.001 | 13.41 |
| -2 log likelihood | 465.7 | | | | |

*****POST C= postmenopausal Caucasian (reference group); PRE C = premenopausal Caucasian; POST SA = postmenopausal South Asian, PRE SA= premenopausal South Asian. ** Body Mass Index. *≠n=83, n=53, n=12 and n=6* in postmenopausal Caucasians, premenopausal Caucasians, postmenopausal Asians and premenopausal Asians respectively. ¥Definition of effect sizes: the absolute value of the quotient of the estimated value and the standard error, Thus defined, the effect size for a parameter is only an indication of how significantly different from 0 the value of the parameter is, i.e. it is an indication of how necessary it is to include, as opposed to excluding, that parameter in the model. The conventional 5% significance level is met for a parameter when the effect size for that parameter meets or exceeds a value of 1.96. However, apart from identifying the importance of including the parameter in the model, the effect size conveys no other information about the functioning of the model.

## Figures

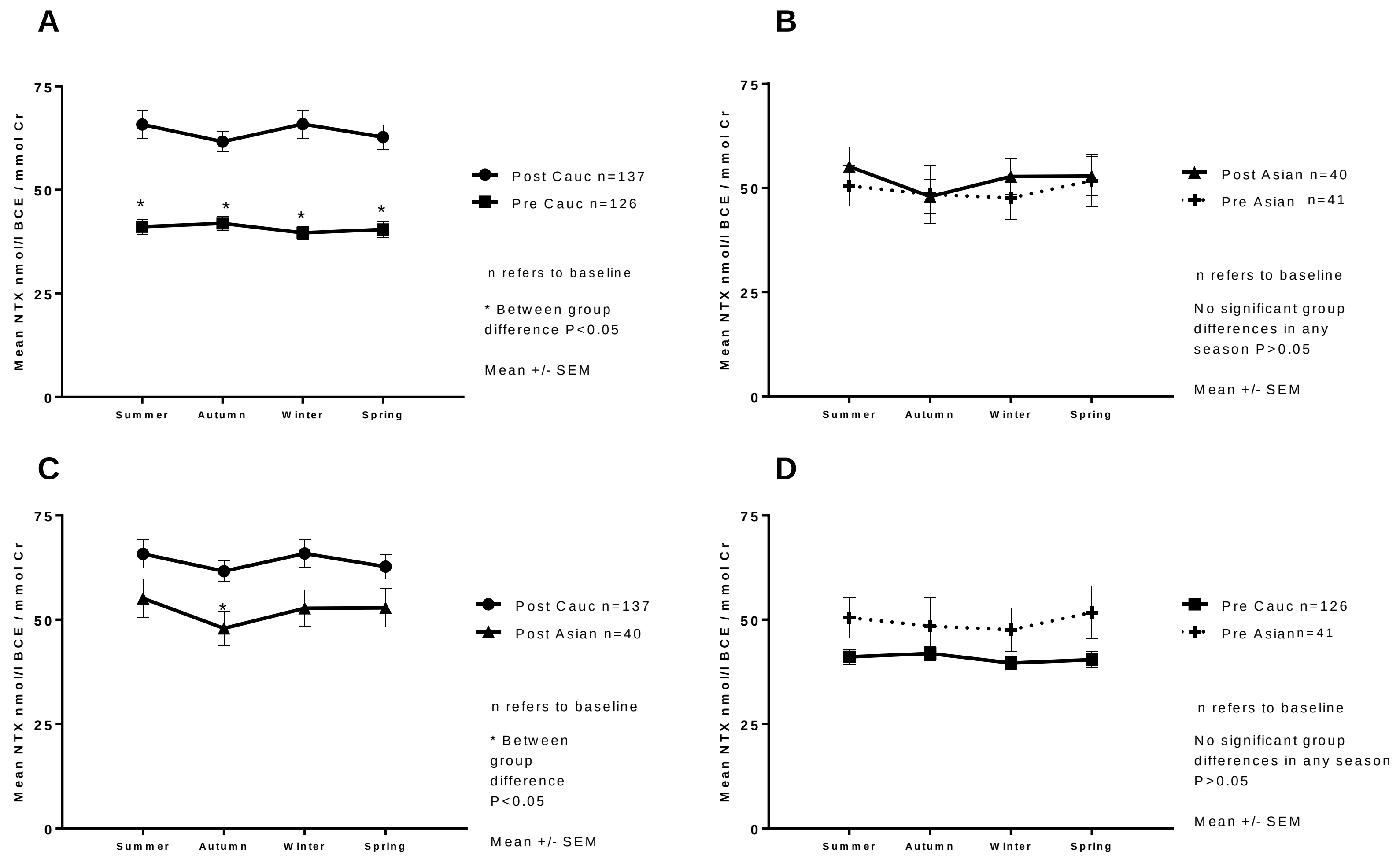


Figure 1 Comparison of unadjusted uNTX data within ethnic groups, women with 1+ seasons (n=344 at baseline (summer)): (A) Two Caucasian groups (B) Two South Asian groups (C) Two postmenopausal Groups (D) Two premenopausal groups. P values derived from within season Independent t-tests.

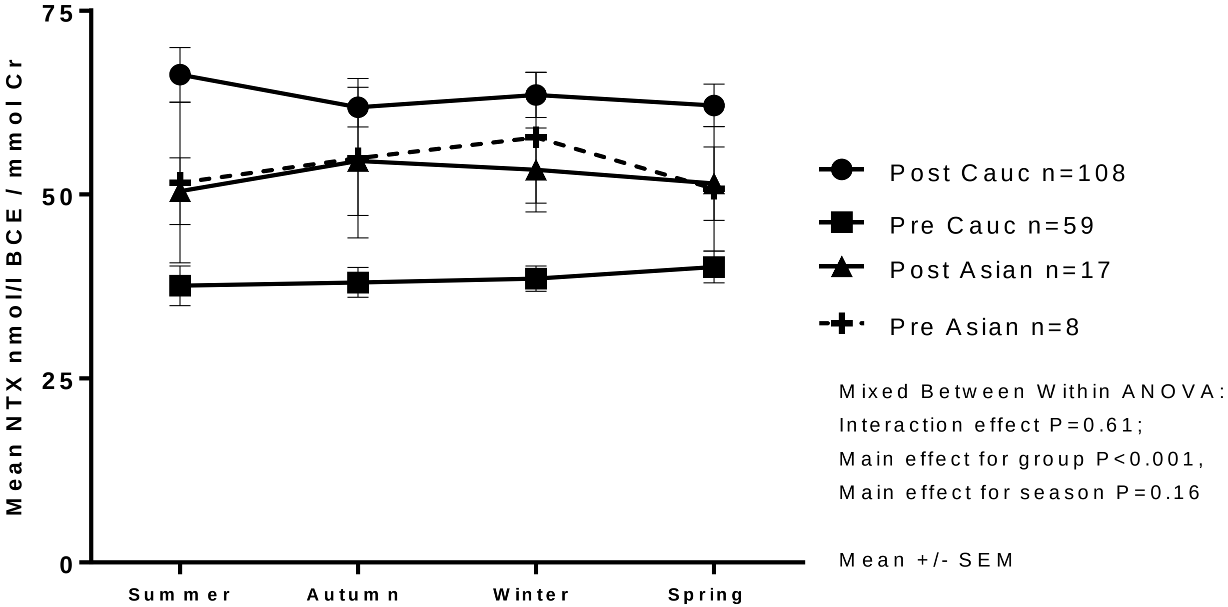


Figure 2 Comparison of all four groups by season- women with full data for uNTX in all 4 seasons only (n=192). Bonferroni adjusted post hoc tests showed the only statistically significant group difference was between the two Caucasian groups (P<0.001).

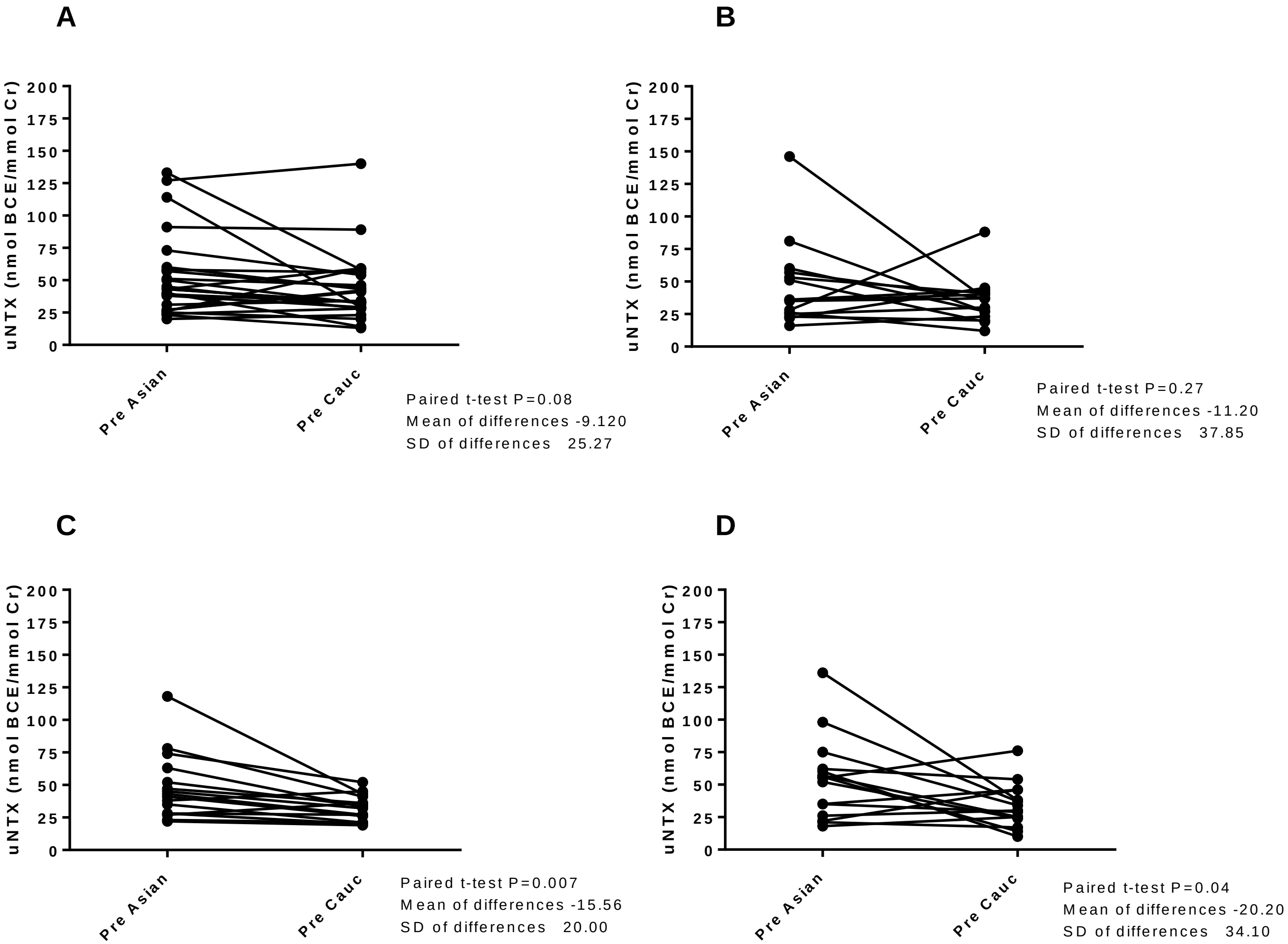


Figure 3 Premenopausal matched pairs (women with uNTX for 1+ seasons) A:Summer (n=25 pairs), B:Autumn (n=15 pairs), C:Winter (n=16 pairs), D:Spring (n=15 pairs)

**Supplementary Table 1: Characteristics of participants in ANOVA analyses (n=192)≠**

| | N | Mean | SD | Lower 95% CI | Upper 95% CI |
|---|---|---|---|---|---|
| Age at start of study (years) | 192 | 52.2 | 12.77 | 50.4 | 54.0 |
| Body Mass Index (BMI) (kg/m$^2$) | 192 | 26.2 | 4.49 | 25.6 | 26.9 |
| Weight (kg) | 192 | 69.0 | 11.07 | 67.4 | 70.6 |
| Height (m) | 192 | 1.6 | 0.07 | 1.6 | 1.6 |
| Dietary calcium (mg)± | 192 | 859 | 269 | 821 | 897 |
| Summer 25(OH)D (nmol/L) | 182 | 62.9 | 26.36 | 59.0 | 66.8 |
| Autumn 25(OH)D (nmol/L) | 179 | 54.7 | 23.87 | 51.1 | 58.2 |
| Winter 25(OH)D (nmol/L) | 166 | 41.4 | 17.65 | 38.7 | 44.1 |
| Spring 25(OH)D nmol/L | 165 | 45.8 | 22.92 | 42.3 | 49.4 |
| Summer uNTX (nmol BCE/mmol Cr) | 192 | 55.5 | 34.35 | 50.6 | 60.3 |
| Autumn uNTX (nmol BCE/mmol Cr) | 192 | 53.6 | 27.11 | 49.8 | 57.5 |
| Winter uNTX (nmol BCE/mmol Cr) | 192 | 54.7 | 28.45 | 50.7 | 58.8 |
| Spring uNTX (nmol BCE/mmol Cr) | 192 | 54.0 | 27.17 | 50.1 | 57.8 |
| | N | Median | 25$^{th*}$ | 75$^{th*}$ | IQR |
| Summer sPTH pmol/L | 182 | 2.7 | 2.1 | 3.4 | 1.3 |
| Autumn sPTH pmol/L | 181 | 2.8 | 2.0 | 3.8 | 1.8 |
| Winter sPTH pmol/L | 163 | 2.8 | 2.1 | 3.7 | 1.6 |
| Spring sPTH pmol/L | 174 | 2.7 | 1.9 | 3.4 | 1.5 |
| | | | | | |
| % South Asian | 13.1 (n=25) | | | | |
| % Postmenopausal | 65.2 (n=125) | | | | |
| Age at Menarche* (mean, SD) y | PostCauc=13(1.6) n=108 P$^{¥}$=0.86<br>PreCauc=13(1.2) n=58<br>PostAsian=13(1.3) n=15<br>PreAsian=13(1.6) n=6 | | | | |
| Age at Menopause* (mean,SD) y | PostCauc=49(5.8) n=67 P$^{≠}$=0.76<br>PostAsian=48(3.6) n=7 | | | | |
| Age at time of study* (2006) (mean,SD) y | PostCauc=61(4.5) n=108 P$^{¥}$<0.001<br>PreCauc$^{a}$=36(4.5) n=59<br>PostAsian=58(4.1) n=17<br>PreAsian$^{a}$=41(4.1) n=8 | | | | |

Cr=creatinine; sPTH=serum parathyroid hormone; sCTX=serum C-telopeptide of collagen; 25(OH)D=serum 25-hydroxyvitamin D; summer to winter 25(OH)D ratio=winter 25(OH)D-summer 25(OH)D; n=number of participants with measurement, ≠ *n*=108,*n*=59, *n*=17 and *n*=8 in postmenopausal Caucasians, premenopausal Caucasians, postmenopausal Asians and premenopausal Asians respectively. ±Dietary calcium was assessed using four-day photograph assisted diet diaries (as previously validated in the EPIC cohort), data shown are baseline (summer) values*percentile, IQR=interquartile range.

**Supplementary Table 2. Characteristics of participants- (n=154) for uNTX non-linear modelling analysis≠**

| | N | Mean | SD | Lower 95% CI | Upper 95% CI |
|---|---|---|---|---|---|
| Age (years) | 154 | 51.5 | 12.7 | 49.5 | 53.5 |
| Body mass index (BMI) ($kg/m^2$) | 154 | 26.1 | 4.6 | 25.3 | 26.8 |
| Weight (kg) | 154 | 68.6 | 11.5 | 66.8 | 70.4 |
| Height (m) | 154 | 1.6 | 0.1 | 1.6 | 1.6 |
| Dietary calcium (mg)± | 144 | 881 | 272 | 836 | 926 |
| Summer 25(OH)D (nmol/L) | 154 | 62.2 | 27.4 | 57.8 | 66.5 |
| Autumn 25(OH)D nmol/L | 154 | 53.9 | 24.5 | 50.0 | 57.8 |
| Winter 25(OH)D nmol/L | 154 | 40.2 | 17.4 | 37.4 | 42.9 |
| Spring 25(OH)D nmol/L | 154 | 45.1 | 23.1 | 41.4 | 48.8 |
| Summer uNTX (nmol BCE/mmol Cr) | 154 | 55.5 | 35.1 | 49.9 | 61.1 |
| Autumn uNTX (nmol BCE/mmol Cr) | 154 | 53.5 | 26.2 | 49.3 | 57.7 |
| Winter uNTX (nmol BCE/mmol Cr) | 154 | 55.0 | 29.3 | 50.3 | 59.7 |
| Spring uNTX (nmol BCE/mmol Cr) | 154 | 54.1 | 27.8 | 49.7 | 58.6 |
| | N | Median | 25th* | 75th* | IQR |
| Summer sPTH pmol/L | 154 | 2.7 | 2.0 | 3.4 | 1.4 |
| Autumn sPTH pmol/L | 153 | 2.6 | 1.9 | 3.7 | 1.8 |
| Winter sPTH pmol/L | 152 | 2.8 | 2.0 | 3.7 | 1.7 |
| Spring sPTH pmol/L | 152 | 2.7 | 1.9 | 3.4 | 1.5 |

| | |
|---|---|
| % South Asian | 12 (n=18) |
| % Postmenopausal | 62 (n=95) |
| Age at Menarche* (mean, SD) y | PostCauc=13(1.6) n=80 P[¥]=0.78<br>PreCauc=13(1.5) n=53<br>PostAsian=13(1.0) n=10<br>PreAsian=13(2.1) n=3 |
| Age at Menopause* (mean,SD) y | PostCauc=49(5.3) n=52 P[≠]=0.93<br>PostAsian=49(3.8) n=5 |
| Age at time of study* (2006) (mean,SD) y | PostCauc=61(4.6) n=83 P[¥]<0.001<br>PreCauc[a]=36(5.1) n=53<br>PostAsian=58(3.2) n=12<br>PreAsian[a]=42 (6.3) n=6 |

Cr=creatinine; sPTH=serum parathyroid hormone; sCTX=serum C-telopeptide of collagen; 25(OH)D=serum 25-hydroxyvitamin D; summer to winter 25(OH)D ratio=winter 25(OH)D-summer 25(OH)D; n=number of participants with measurements. *n=83, n=53, n=12 and n=6* in postmenopausal Caucasians, premenopausal Caucasians, postmenopausal Asians and premenopausal Asians respectively ±Dietary calcium was assessed using four-day photograph assisted diet diaries (as previously validated in the EPIC cohort) *percentile, IQR=interquartile range

**Supplementary Figure 1. Flow of participants through the statistical analyses in the manuscript**